LABORATORY SCREENING STUDIES ON THE BIODEGRADATION OF ORGANICS IN RMA GROUND WATER

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ABSTRACT

Data is presented on the potential for *in situ* bioremediation of organic compounds in ground water at RMA sites. The laboratory results demonstrate that 1) ground water microcosms containing aquifer cores from Basin A Neck can be used to assess biodegradation, and 2) benzene and chloroform (50 ppb - 50 ppm) can degrade at significant rates (2 - 20%/day) in the presence of inorganic nutrients (NH_4^+ and PO_4^{-3}) and dissolved oxygen ($\geq$ 2 ppm). Similar laboratory experiments in soil microcosms are planned to investigate the bioremediation potential for these and other compounds (chlorobenzene, dicyclopentadiene, and bicycloheptadiene) present in the South Tank Farm plume using actual aquifer cores from that plume.

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by

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INTRODUCTION

An examination of the literature on the biodegradation of aromatic hydrocarbons such as benzene (B), toluene (T), xylene (X), and chlorobenzene (CB) indicates that these are readily metabolized by a variety of naturally-occurring bacteria and fungi in soil[1]. Initial enzymatic attack on the ring is through cis-diol formation (oxygenases), followed by ring opening and oxidation to a dicarboxylic acid derivative. This acid is further metabolized to CO_2 through several pathways (e.g., citric acid cycle) in the cell. Table 1 gives a summary of literature on soil biodegradation of some compounds which have been observed in RMA ground water samples.

BTX concentrations of 50 - 5000 ppb are rapidly depleted at rates of 2 - 70%/day in laboratory soil/ground water microcosm test systems or bacterial cultures isolated from soil. These rates are, however, significantly higher than those obtained (ca. 1%/day) from field estimations using plume modeling techniques.

Recent evidence by Baker et al.[2] and Chiang et al.[3] suggests that the field estimated decays for aromatic hydrocarbons is highly dependent upon the dissolved oxygen (DO) concentration in the ground water and its reaeration rate (via upstream aquifer flow and rainfall recharge events). Nevertheless, these data indicate that subsoils have the capacity to biodegrade high levels of organic compounds and factors such as DO levels, nutrient (NH_4^+, PO_4^{-3}) availability and diversity of subsoil microbial populations are probably important.

Chlorobenzene is most likely degraded similarly to benzene[1] and biotransformation rates of 0.4 - 14%/day have been observed with soil and

sewage isolates.[6,7,8] There are reports on the biodegradability of chloroform in soil in which 0.3 and 500 ppm was metabolized at rates of 2.5 - 14%/day.[9,10] No literature data exists on the degradation (soil or microbial cultures) of the cycloalkanes, dicyclopentadiene and bicycloheptadiene, however, they may be metabolized similarly to the cyclodiene insecticides, dieldrin, aldrin, and endrin in which an epoxide, alcohol, ketone or carboxylic acid derivative could be formed.[11]

The following report describes some preliminary experiments on the biodegradation of benzene and chloroform in RMA ground water. Test tube microcosms were used to evaluate the degradation potential of these compounds in aquifer sediments isolated from the Basin A Neck area at the RMA site. These aquifer sediments are considered representative for measuring biodegradation potential of subsurface sediments and groundwater at the RMA site.

MATERIALS & METHODS

Aquifer Sediment Samples. Borings in the saturated zone around Basin A Neck at RMA were obtained by Morrison Knudsen Environmental Services (MKE) staff using a Waterloo saturated sand sampler described previously by Zapico et al.[12] Polybutyrate Shelby tubes (sterilized with bleach solution), measuring 3 inches by 5 feet were inserted into the stainless steel wireline piston core barrel ("Waterloo sampler"). A hollow stem auger was used to make initial boreholes just below the saturated zone of Basin A neck (Northeast Section 35). At these depths, the auger was replaced by the sampler, forced into the saturated zone and 2 foot aquifer cores retrieved. The samples were designated BANIBR-B (14.2 - 31.5 ft) placed on ice and shipped to Westhollow Research Center (WRC) for use in microcosms. The sample was from an uncontaminated area outside the Basin A plume.

Microcosm Test System. Ground water/subsoil test tube microcosms were used to simulate subsurface conditions in the degradation of benzene and chloroform. Microcosms were prepared by aseptically transferring 10g of inner core (14.2 - 16.2 ft sample) of soil from samples in polybutyrate tubes to sterile serum-stoppered test tubes fitted with Teflon®-lined rubber septa and aluminum crimp seals. Uncontaminated ground water from developed

wells was added (25 - 27ml) to microcosms and the DO's adjusted to 2 or 8 ppm by flushing with N_2 or air for varying periods. DO measurements were made with an Orbisphere Model 2111 BOD probe and Model 2607 meter. Sterile sediment slurries were used as controls and contained gamma-ray irradiated (7.5 Mrad, Neutron Products, Inc., Dickerson, Maryland) soils and filter-sterilized (0.22 micron Millipore) ground water. Duplicate sterile and non-sterile microcosms were prepared for benzene (5, 50 ppm) and chloroform (50, 500 ppb) concentrations. Slurries were placed in an anaerobic glove box (Vacuum Atmospheres Company, Hawthorne, California) containing an inert N_2 ($\leq$ 1 ppm O_2) atmosphere. Inorganic nutrients (N & P) and trace minerals were added to microcosms at final concentrations of 5 - 10 ppm NH_4^+ and NO_3-N and PO_4-P and 0.3-1 ppm Ca^{++} and Mg^{++}. All test slurries were incubated at 12 - 15°C in the anaerobic chamber.

Microbial & Chemical Analyses. Microbial populations in soil and ground water were enumerated by tube extinction dilution methods. Endpoints of serial 1:10 dilutions of soil made into culture media were the highest dilution showing turbidity or microscopically discernible cell growth (aerobes, facultative anaerobes or anaerobes), FeS precipitation from H_2S production (sulfate-reducers), methane formation (methanogens) or no residual NO_3^- (denitrifers). Aerobes were cultured in Trypticase Soy Broth (BBL, Becton, Dickinson & Company) for tube dilution or on R_2A medium[13] for colony counts. Denitrifers, sulfate-reducers and methanogens were estimated by culturing in denitrifying medium (0.8% Difco Nutrient Broth, 0.1% $NaNO_3$ and 0.5% succinate), modified Postgate medium E[14] with 0.1% sodium acetate and Balch medium I[15], respectively. Cultures were incubated at 30°C for 7 - 14 days.

At each sample point, the aqueous portion of microcosms was removed with a needle and syringe inside the anaerobic chamber and one ml sample placed into vials with no headspace. Samples were analyzed for benzene and chloroform by purge and trap gas chromatography[16] methods using a Tekmar purge and trap apparatus and Varian GC (1 and 5 ppb minimum detection level for benzene and chloroform, respectively). This is essentially Method 602 proposed by the U.S.E.P.A. for volatiles in water samples.

RESULTS & SUMMARY

Subsoil Analyses - Physical-Chemical Properties & Microbial Populations. Analyses of four saturated soil cores taken from depths of 14 - 31 ft are given in Table 2 and indicate some heterogeneity in the amount of sand, silt, and clay, with clay comprising 20 - 35%. The amount of available N (NH_4^+ and NO_3^-) and P (PO_4^{-3}) also varied slightly from 3 - 7 ppm N and 15 - 25 ppm P among the cores at different depths. The samples contained low levels of soluble Fe (1 - 8 ppm) but high concentrations of total Fe (1.4 - 1.6%). These levels of available N and P would probably represent sufficient requirements for microbes to degrade 0.005 - 50 ppm benzene and chloroform in ground water (based on a balanced C/N/P cell ratio 25/5/1).

Total microbial populations of aerobes and facultative anaerobes is given in Table 3. The number of aerobic bacteria varied from 10^3 - 10^8/g wet wt soil and appeared to increase with depth. Facultatively anaerobic bacteria were fewer in numbers (10^3 - 10^4/g) while more strict anaerobic species (denitrifiers, sulfate-reducers, and methanogens) were essentially non-detectable. The data show that these RMA aquifer soil cores contain predominantly aerobic organisms and that any degradation potential would probably be due to aerobic metabolism.

Biodegradation of Benzene (B) & Chloroform (CF) in Aquifer Cores. Data on the potential of laboratory microcosm systems for estimating the biodegradation of B and CF in ground water are given in Figures 1 & 2 and summarized in Table 4. These represent the degradation profiles in the subsoil incubated at 12 - 15°C in the presence of 5 and 50 ppm of B, and 50 and 500 ppb CF at DO levels of 2 or 8 ppm. The biodegradation of benzene was more rapid at 50 ppm (20%/day) than at 5 ppm (2%/day) in the presence of added nutrients (NH_4^+-N and PO_4^{-3}-P) and oxygen (DO, 2 or 8 ppm) and were substantially higher than the sterile control depletions.

It is not clear why the B depletion rate at 5 ppm is less than that at 50 ppm, but it may indicate 1) an unusually high nutrient requirement for low concentrations (not present in the experiment) or 2) that there is significant heterogeneity in microbial metabolism between soil core samples or 3) that there is significant soil sorption effects of B at

low concentrations. This effect needs to be confirmed with additional microcosm and nutrient experiments.

The biodegration of CF at 50 and 500 ppb in ground water indicates that the rates were similar (5 - 10%/day). Although some variability was observed between duplicate microcosms (A & B) these decreases in CF were observed in the presence or absence of added nutrients and at an initial DO of 2 or 8 ppm. The potential for CF to degrade in aerobic ground water, however, may be a new finding since it has been shown that many chlorinated alkanes are primarily metabolized under strict anoxic conditions (absence of detectable O_2) by anaerobic bacteria via a reductive dehalogenation[17] or aerobically in the presence of methane (as alternate co-substrate) to enhance the growth of methanotrophic bacteria in soil.[18]

FUTURE STUDIES

Additional experiments are planned with South Tank Farm aquifer cores to further investigate the limits and potential for the aerobic biodegradation of benzene and chloroform plus other ogranics, such as, chlorobenzene, dicyclopentadiene, and bicycloheptadiene. We expect the biodegradation of benzene to be similar to that observed with Basin A Neck aquifer sediments and groundwater, as well as, at other sites around the country as mentioned in the Introduction section.

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TABLE 1: SUMMARY OF LITERATURE DATA ON AEROBIC BIODEGRADATION IN SOIL

COMPOUND	CONCENTRATIONS TESTED, ppm	TEST SYSTEM[a)]	DEGRADATION %/DAY	REFERENCE
Benzene	1.8 - 2.4	Soil/GW (field)	0.7 - 1.1	2,3
	0.05 - 5	Soil/GW	1.8 - 20	2,3
Toluene	1.8 - 2.6	Soil/GW (field)	1.4	2
	0.05 - 5	Soil/GW	2.5 - 20	3
Xylenes	0.05 - 5	Soil/GW	2.5 - 70	2,3,4
BTX	1 - 56	Various cultures	121.5	5
Chlorobenzene (CB)	1	Soil	1.6	6
	20	Soil	0.4	7
	660	Soil/sewage isolate	14	8
	20	Nocardia/ Pseudomonas	6 - 10	7
Chloroform	500	Soil	2.5	9
	0.30	Soil (Methane-stimulated)	14	10
Dicyclopentadiene (DCPD)	___[b)]	___	___	___
Bicycloheptadiene (BCH)	___	___	___	___

a) Most were laboratory test systems unless otherwise indicated; GW = groundwater.

b) ___, no data available.

TABLE 2: SUBSOIL CHARACTERISTICS

	DEPTH (FT)			
PARAMETER	14.2 - 16.2	19.5 - 21.5	24.5 - 26.5	29.5 - 31.5
pH	8.1	7.5	7.8	8.3
SAND/SILT/ CLAY (%)	73/8/19	54/11/35	70/5/25	64/9/27
CLASS	SL	SC	SCL	SCL
% FINES <0.1mm	59	43	42	46
AVAILABLE NUTRIENTS (ppm)				
NH_4-N	4.8	5.7	4.1	2.7
NO_3-N	2.8	.4	.7	.7
PO_4-P	25	16	20	21
SOLUBLE Fe (ppm)	8.1	1.1	1.6	2.5
TOTAL Fe (ppm)	14700	13860	15020	16410

OTHER PROPERTIES - range for all depths

SOLUBLE ANIONS AND CATIONS (ppm)
- CL 3.8 - 5.1
- SO_4 8.1 - 23.2
- Na-K 229 - 327
- Ca-Mg 4.3 - 13.2

BULK DENSITY (g/cc) 1.26 - 1.36

PARTICLE DENSITY (g/cc) 2.52 - 2.75

* Analyzed by Soil Analytical Services, Inc., College Station, Texas, for BANIBR cores.

TABLE 3: BACTERIAL POPULATION DISTRIBUTION IN SEDIMENTS[a)]

SAMPLE DEPTH (FT)	NUMBER OF BACTERIA/GRAM WET. WT.[b)] AEROBES TUBE DILUTION	AEROBES PLATE COUNT	FACULTATIVE ANAEROBES
	X 10^5		
14.2 - 16.2	0.1 - 1	0.2 - 0.3	0.1
19.5 - 21.5	.01 - .1	.08 - 1	.01 - 1
24.5 - 26.5	100	50 - 80	.01 - 1
29.5 - 31.5	100 - 1000	30 - 100	.1

a) BANBR sample (NE Section 35, Basin A Neck).

b) Number of denitrifiers, sulfate reducers and metanogens were < 100/g (min. detection level).

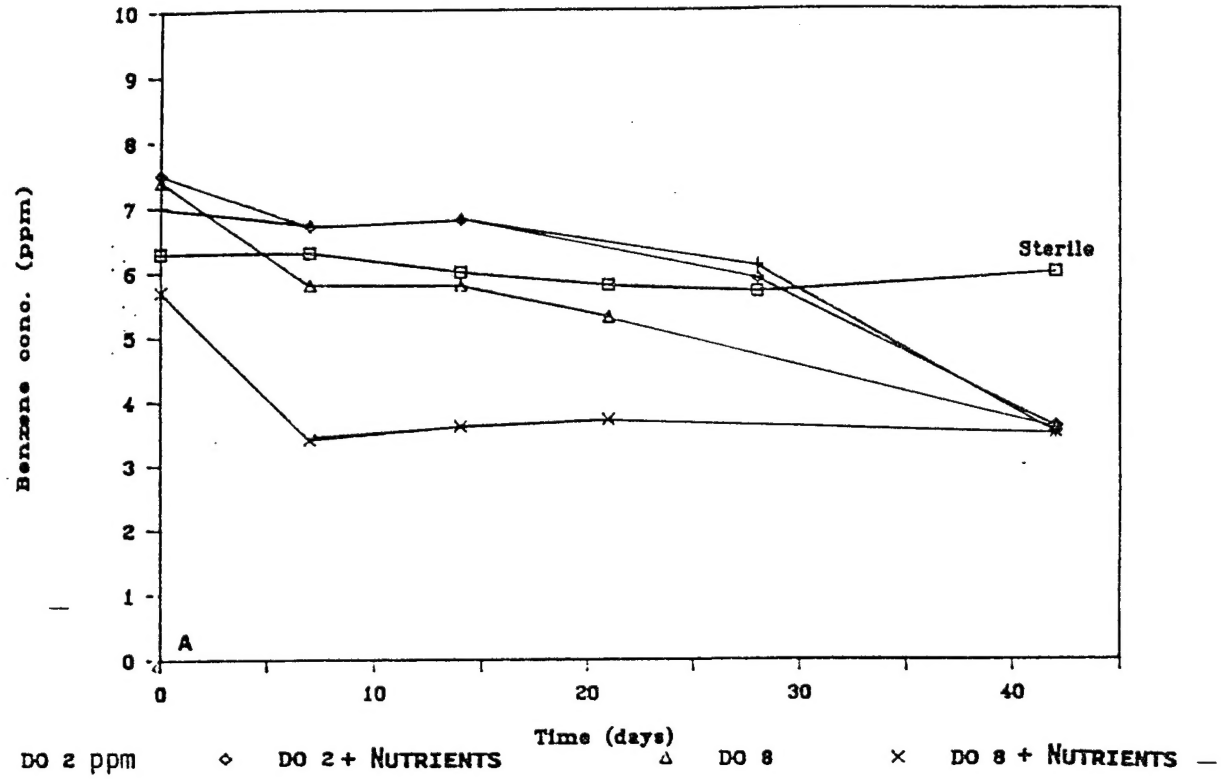
TABLE 4: SUMMARY OF GROUND WATER MICROCOSM RESULTS

COMPOUND (concn)	% DEGRADATION DEGN/DAY[a)]	COMMENTS
Benzene (50 ppm)	20	DO, 8 ppm, + nutrients[b)]
(5 ppm)	2	DO, 2 or 8 ppm, ± nutrients
Chloroform (500 ppb)	5-8	DO, 2 or 8 ppm, ± nutrients
(50 ppb)	11	DO, 2 ppm, ± nutrients

a) Assumes a first-order decay, rate = $\ln(Co/Ci)/t$

b) Effects observed at dissolved oxygen (DO) levels given and ± nutrients (N, NH_4^+/NO_3^-; P, PO_4^{-3})

FIGURE 1: BIODEGRADATION OF BENZENE AT 5.5 - 7.5 PPM (A) AND 55 - 65 PPM (B) IN RMA AQUIFER SOIL AND GROUND WATER



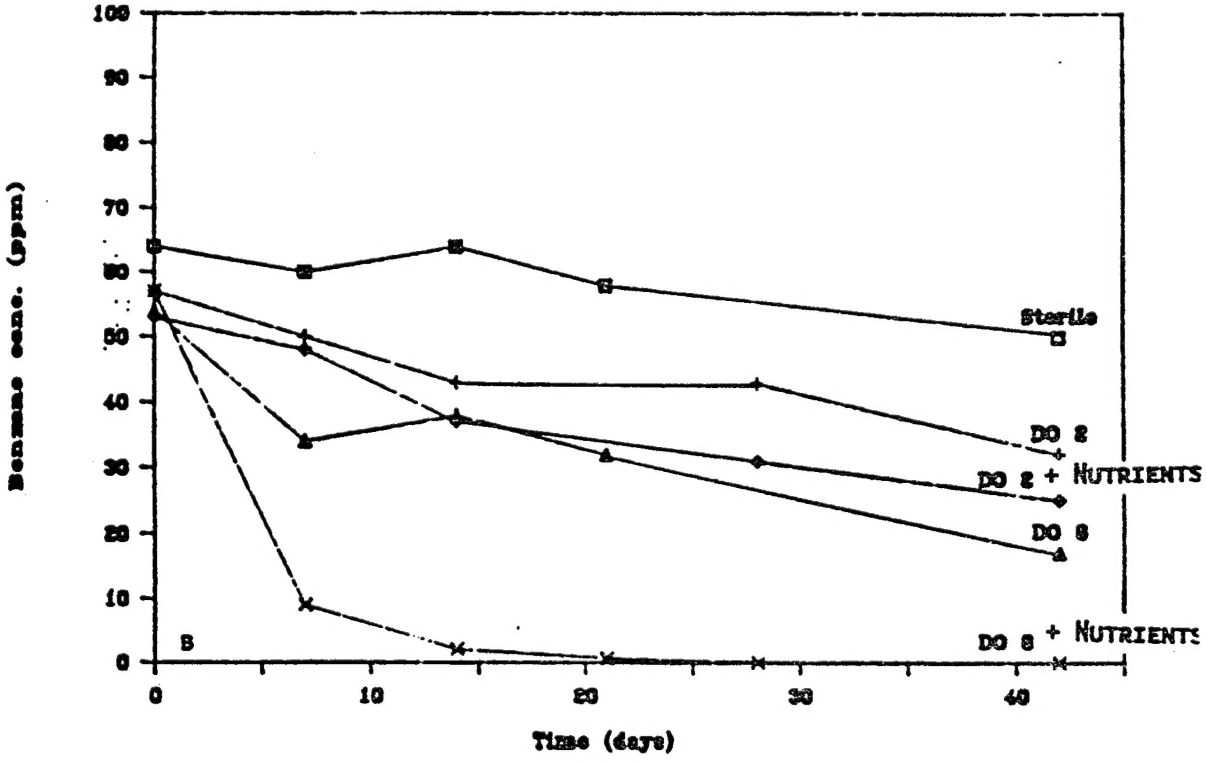
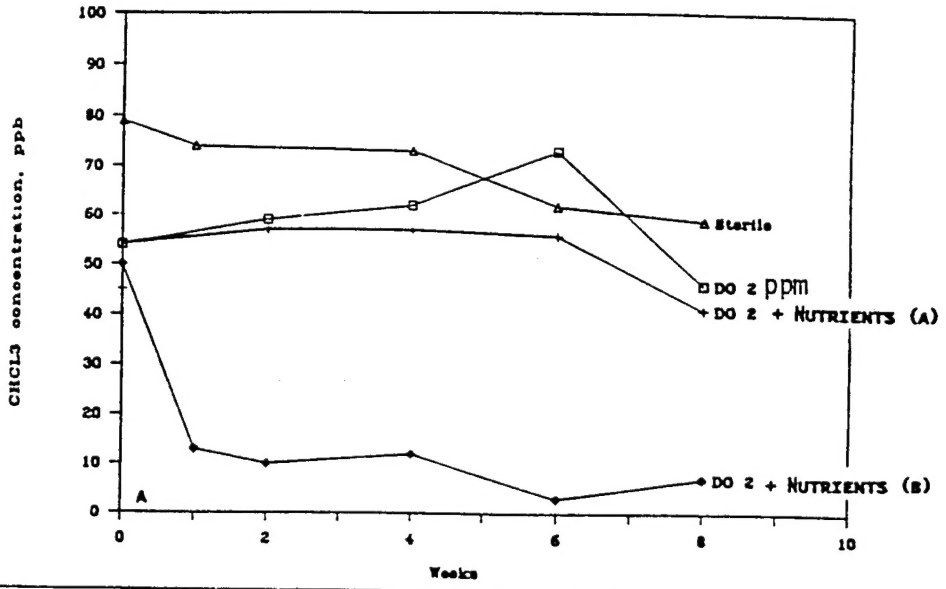
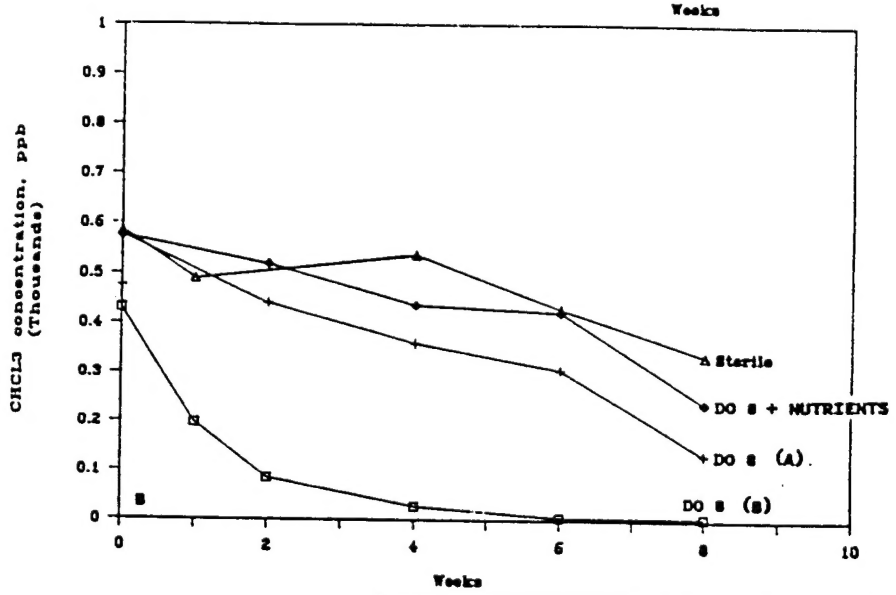
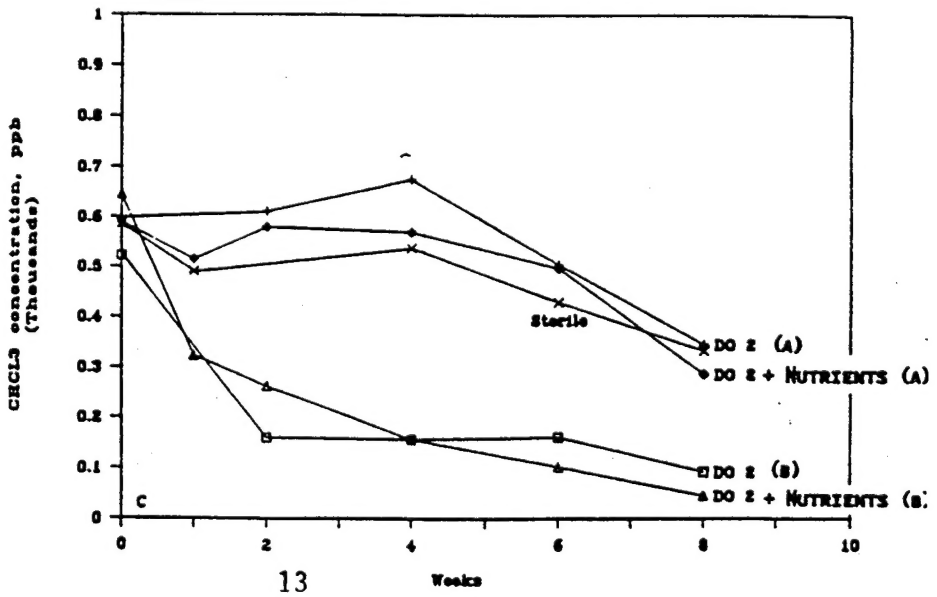


FIGURE 2: BIODEGRADATION OF CHLOROFORM AT 50 - 80 PPB (A) AND 450 - 650 PPB (B & C) IN RMA AQUIFER SOIL AND GROUND WATER







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THIS REPORT DESCRIBES PRELIMINARY EXPERIMENTS ON THE BIODEGRADATION OF C6H6 AND CHCL3 IN RMA GROUND WATER. TEST TUBE MICROCOSMS WERE USED TO EVALUATE THE DEGRADATION POTENTIAL OF THESE COMPOUNDS IN AQUIFER SEDIMENTS ISOLATED FROM THE BASIN A NECK AREA.

THE LABORATORY RESULTS DEMONSTRATE THAT:

1. GROUND WATER MICROCOSMS CONTAINING AQUIFER CORES FROM BASIN A NECK CAN BE USED TO ASSESS BIODEGRADATION
2. C6H6 AND CHCL3 CAN DEGRADE AT SIGNIFICANT RATES IN THE PRESENCE OF INORGANIC NUTRIENTS (NH4 AND P04).

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